

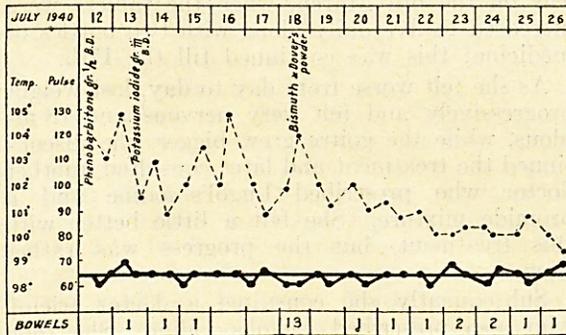
There was nothing particular with regard to her family and personal history.

Physical examination.—A thin under-nourished woman with a staring look; an average degree of exophthalmos with indefinite eye signs of Graves' syndrome; moderate-sized thyroid enlargement (maximum circumference of the neck—13½ inches) with smooth surface and definite systolic bruit; a rather excitable heart with a pulmonary systolic bruit; a collapsing type of pulse with a regular rate of 120 per minute; markedly pulsating abdominal aorta; fine tremor of the tongue and out-stretched fingers and moist warm palm. Her weight was 132 pounds; she had lost four stone in five months. The blood pressure was systolic 125 and diastolic 55 mm. of Hg., with a pulse pressure of 70. Following Read's formula— $0.75 (\text{pulse rate} + 0.74 \times \text{pulse pressure}) - 72$ —the basal metabolic rate was plus 56.8 per cent. The B. M. R. actually estimated by the Benedict-Roth metabolism apparatus was plus 50 per cent.

Blood count.—Hæmoglobin 68 per cent = 9.35 grammes per 100 c.cm.; reticulocytes—0.2 per cent; red cells—3,620,000 per c.mm.; cell volume—30 per cent; mean corpuscular volume 83.3 cu μ ; mean corpuscular hæmoglobin 25.9 $\gamma\gamma$; mean corpuscular hæmoglobin concentration 31 per cent; leucocytes—6,000 per c.mm.; polymorphonuclears—34 per cent; lymphocytes—55 per cent; large mononuclears—6 per cent; and eosinophils 5 per cent.

Van den Bergh test—negative.

Other laboratory findings are not relevant.



Treatment.—The patient was at once made a bed-case and put on phenobarbitone, grain ½ twice a day. Next day she was given potassium iodide, grains iii twice a day along with a high calorie diet. The iodide did not upset her in any way. After about a week while she was beginning to settle down, it was suggested that she might require an operation. This was enough to upset her nerves and caused severe diarrhœa—thirteen watery stools in twenty-four hours, which was readily controlled with two doses of bismuth and Dover's powder. The condition gradually improved and pulse came down to 72 per minute on the 15th day

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SULPHATHIAZOLE IN SOME EXPERIMENTAL BACTERIAL AND VIRUS INFECTIONS

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This paper presents the results of testing sulphathiazole in experimental *Bacterium typhosum*, *Vibrio cholerae* and fixed-virus infections in mice, and vaccinia-virus infection in

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although she had further lost in weight. Subsequently she agreed to be operated upon and was transferred to the surgeon for preparatory treatment and thyroidectomy.

Summary and discussion

A woman, apparently with Graves' constitution, accidentally discovered one day that her thyroid was swollen. There was no obvious evidence of general or focal infection, or nervous shock that is usually regarded as the provocative factor in the causation of Graves' disease.

It is possible, there might have been psychic trauma which was difficult for the patient to recall to memory; or it might have been that she had undergone such a trauma but that its nature was distasteful to narrate. In the absence of careful psycho-analysis by experts no further light could be thrown on the subject.

Very likely the patient had the thyroid hyperplasia for some time before she happened to discover it, and she was obviously sensitive to the effects of thyroid, so that the condition was aggravated to a marked thyrotoxic state by its injudicious administration in increasing doses.

Discontinuation of the drug and treatment with rest, Lugol's iodine and sedatives improved her condition, but later she used to get sick with the iodine solution.

During a subsequent exacerbation she was admitted in the Carmichael Hospital for Tropical Diseases. The clinical picture has been described and the result of basal metabolic rate, as determined by Read's formula as well as gasometric method given. Blood count showed an increase in the percentage of lymphocytes.

Treatment consisted of bed-rest, potassium iodide in small doses (this was well tolerated by the patient), phenobarbitone and high calorie diet. The progress was satisfactory, and there was a steady improvement except one attack of typical nervous diarrhœa of thyrotoxic origin.

She was subsequently handed over to the surgeon.

Acknowledgment

My grateful thanks are due to Dr. L. E. Napier for his kind permission to publish this note and for valuable suggestions.

rabbits. For comparative study sulphanilamide and sulphapyridine were also tested in some infections.

Bact. typhosum

A strain isolated from blood of a severe case of typhoid was used. This organism, immediately after isolation, was not found to be very virulent for mice. After nine passages in mice it gained considerable virulence; 100 million organisms given intra-peritoneally produced death in 24 hours. The mice used in the experiments were of the Haffkine Institute in-bred strain, 6 weeks old, and weighing between 15 and 20 grammes. Older mice were found to be less susceptible to infection. For purposes of experiment, 100 million organisms from a 17-hour-old culture of the organism in ordinary nutrient broth were injected intra-peritoneally into mice. The drug under test was made into an emulsion in gum acacia so that 0.5 c.cm. of this emulsion contained the required amount of the drug. This quantity was fed to the mouse by means of a glass pipette introduced into its stomach. The results are shown in table I.

TABLE I

The therapeutic effect of sulphanilamide, sulphapyridine and sulphathiazole in experimental Bact. typhosum infection in mice. The drugs were administered in 20 mg. doses soon after infection, 10 hours later and then twice daily for 3 more days

Drug administered	Number of mice out of a group of 16 dying on each day after infection						Survivors	Average survival time (days: max. 10)
	1	2	3	4	5	6-10		
Sulphanilamide	4	12	7.5
Sulphapyridine	3	2	11	7.3
Sulphathiazole	2	1	..	2	11	7.3
Controls	16	0	0.0

V. cholera

An Inaba strain of *V. cholera* was passed serially through 6-weeks-old mice until it was found to have acquired sufficient virulence for

mice. It was found that after eleven passages about 875 million vibrios killed mice in 24 hours. Further passages however did not enhance the virulence. For purposes of experiment 875 million vibrios from a 17-hour-old culture were injected intra-peritoneally into 6-weeks-old mice. The drug was administered as in the experiment with *Bact. typhosum*. The results are shown in table II.

TABLE II

The therapeutic effect of sulphanilamide, sulphapyridine and sulphathiazole in experimental V. cholera infection in mice. The drugs were administered in 30 mg. doses soon after infection and 10 hours later

Drug administered	Number of mice out of a group of 12 dying on each day after infection						Survivors
	1	2	3	4	5	6-10	
Sulphanilamide	12	0
Sulphapyridine	12	0
Sulphathiazole	12	0
Controls	12	0

Bacteriostatic effect on V. cholera

In view of the absolute lack of therapeutic effect of the drug in *V. cholera* infection, an 'in vitro' test was put up to ascertain if the drug possessed any bacteriostatic properties in relation to *V. cholera*. A test with sulphanilamide was also put up for comparison. The results are shown in table III.

Fixed rabies virus

The strain of fixed rabies virus (Paris strain) used in the routine manufacture of antirabic vaccine in this institute was used for purposes of infection. Three-weeks-old mice were used as they give uniform results. The minimum infective dose of the virus as determined by the method of Webster (1939) was 0.03 c.cm. of 1/160 dilution of the virus. The virus was diluted in 10 per cent horse serum in distilled water and 0.03 c.cm. of a 1/40 (representing four

TABLE III

Bacteriostatic action of sulphanilamide and sulphathiazole on V. cholera

Drug	Number of organisms inoculated per c.cm.	Number of hours that elapsed between inoculation and appearance of growth in the various dilutions of the drug				
		1-2,500	1-5,000	1-10,000	1-20,000	1-30,000
Sulphanilamide	{ 500	48	24	24	24	24
	{ 5,000	48	24	24	24	24
Sulphathiazole	{ 500	168	144
	{ 5,000	120	96	48
Sulphathiazole + para-aminobenzoic acid	{ 5,000	Not tried	Not tried	24	24	24

minimum infective doses) dilution of the virus was injected intramuscularly into the lower third of the gastrocnemius muscle of the mouse. Sulphathiazole was administered as in the previous experiments. The result was a complete failure of the drug in checking the fixed virus infection. All the mice died within 15 days of paralysis.

Vaccinia virus

Vaccine lymph obtained from the vaccine lymph institute at Belgaum and white rabbits weighing between 1.25 and 1.5 kg. were used in these experiments. The backs of the rabbits were shaved and about 0.1 c.cm. of vaccine lymph was smeared over the shaved surface which was then lightly 'scarified'. The drug was administered to one rabbit by mouth. Two received the solution of sodium salt of the drug parenterally and two served as controls. The results are shown in table IV.

TABLE IV
The therapeutic effect of sulphathiazole in vaccinia virus infection in rabbit

Weight of rabbit	Mode of treatment	Result
1.34 kg.	1 g. by mouth twice daily for 3 days.	Moderate 'take' after 72 hours.
1.32 kg.	0.375 g. (Na salt) intramuscularly twice daily for 3 days.	Do.
1.35 kg.	Do.	Do.
1.48 kg.	Control—not treated	Typical 'take' in 72 hours.
1.52 kg.	Do.	Do.

Discussion

Buttle *et al.* (1937) first reported that sulphanilamide, administered immediately after infection in single or repeated doses of 25 mg. protected a large percentage of mice against a moderate infecting dose of *Bact. typhosum*. Powell and Chen (1939) found that sulphanilamide in two doses of 5 mg. gave a good protection against 10 to 100 lethal doses of *Bact. typhosum*, while sulphapyridine under the same conditions gave inferior results. Kolmer and Rule (1939), who injected the above two drugs in doses of 0.160 g. per kg. body-weight, obtained only poor results, sulphapyridine being a little inferior to sulphanilamide. In our experiments (table I) there was no significant difference in therapeutic effect between sulphanilamide, sulphapyridine, and sulphathiazole. Though the protection given by these drugs in this experimental infection cannot be considered to be as spectacular as that obtained in the case of streptococcal or pneumococcal infections, the results are striking enough to justify a careful clinical trial with these drugs. The clinical data reported so far contain some favourable reports of treatment

with prontosil, sulphanilamide, and sulphapyridine in typhoid fever, persistent typhoid bacilluria and pyelitis, typhoid caries, etc., and also a few unfavourable ones; but these are too scanty to admit of a proper evaluation of the efficacy of these drugs in this infection (cf. Buttle, 1939; Kolmer, 1940). Very recently, good results are reported by Weilbaecher *et al.* (1940) in 3 of 4 patients treated with sulphathiazole. To obtain the best results, it is important that the treatment with these drugs should be instituted as early as possible and an adequate blood concentration of about 5 mg. per cent of the drugs maintained throughout the course of the infection.

Table II clearly shows sulphanilamide, sulphapyridine or sulphathiazole to be of no value in cholera. This negative result is of interest in evaluating the validity of some of the theories proposed regarding the mechanism of action of this class of drugs. Locke and Mellon (Mellon *et al.*, 1940) have advanced the anti-catalase theory which postulates that the therapeutic effect of the sulphanilamides is due to the injury caused to the organisms by hydrogen peroxide accumulated at the focus of infection as a result of the inactivation of the enzyme, catalase, by the drug. This theory requires that the organisms which produce catalase and are sensitive to injury by hydrogen peroxide should, as a class, be amenable to treatment by these sulphanilamides. But the case of *V. cholerae* apparently throws doubt on the validity of this theory for this microbe has been shown by M'Leod and Gordon (1923) to be markedly sensitive to hydrogen peroxide and also to produce catalase though to a small extent. Recently, Woods (1940) and Fildes (1940) have advanced the theory that the sulphanilamides act by inhibiting the action of an enzyme of some fundamental importance by competing with its substrate which is an 'essential metabolite' surmised to be *para*-aminobenzoic acid. According to this theory, the sensitiveness of a microbe to sulphanilamide would depend upon whether it could synthesize this substrate readily or not, *i.e.*, a microbe that is not sensitive to the drug should be able to synthesize *para*-aminobenzoic acid in excess to overcome the inhibition by sulphanilamide. In the case of *V. cholerae*, we find a marked discrepancy between the *in vivo* and the *in vitro* results. As shown in table II, sulphathiazole shows absolutely no protective power against this micro-organism in mice. But *in vitro*, this drug possesses a striking bacteriostatic effect even in a dilution of 1 : 30,000 and this action is reversed by the addition of *para*-aminobenzoic acid, which by itself possesses no perceptible growth-stimulating effect on *V. cholerae*. This behaviour of the organism *in vitro* is not distinguishable from that of the streptococci or pneumococci, both of which respond to treatment with sulphathiazole. It is difficult to reconcile this difference in behaviour of sulphathiazole towards *V. cholerae* between

the *in vivo* and *in vitro* experiments, in the light of the theory of Woods and Fildes unless one assumes that this organism produces *para*-aminobenzoic acid in excess *in vivo*, while it is not able to do so *in vitro*. It is doubtful whether this is the case and further experiments are in progress to examine this question.

Till now, the action of the sulphanilamides have not been tested in experimental vaccinia virus infections but there are a few instances on record (McCammon, 1939; King and de Rozario, 1938) of the beneficial effects of prontosil and sulphanilamide in smallpox. The results recorded in table IV indicate that the drugs do not possess any specific therapeutic effect against this virus and obviously the above-mentioned favourable clinical results are due to the prevention of the secondary streptococcal infection of the lesions by these drugs. The sulphanilamide derivatives may thus be of use in smallpox, if at all, only in this direction.

The action of sulphanilamide and some of its derivatives in experimental rabies infections have previously been reported. Kirk (1939) has found 'prontosil' to have no effect on rabies of rabbits. McCrea (1939) has reported that sodium sulphanilyl-sulphanilate prevented the development of rabies in one-third of the number of rabbits; this result does not appear to be very definite. Gross, Cooper and Lewis (1939) have found only a very slight prolongation in life of rats infected with the virus when treated with sulphanilamide or sodium sulphanilyl-sulphanilate. Powell and Chen (*loc. cit.*) have declared sulphanilamide and sulphapyridine to be of no value in rabies in mice. Our results show that sulphapyridine and sulphathiazole also are of very little value in rabies of mice.

Summary

1. The therapeutic value of sulphathiazole in experimental *Bact. typhosum*, *V. cholerae* and fixed rabies virus infection in mice and vaccinia virus infection in rabbits has been investigated.

2. Experimental results show that the drug has appreciable therapeutic effect in experimental *Bact. typhosum* infection in mice and is of no therapeutic value in experimental *V. cholerae* and fixed rabies virus infection in mice and vaccinia virus infection in rabbits.

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AGES OF EPIPHYSIAL UNION AT ELBOW AND WRIST JOINTS AMONGST 238 CHILDREN IN NORTH WEST FRONTIER PROVINCE

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VERY frequently doctors are called upon to give an opinion as to the age of a person, either for medico-legal purposes, for entry to government service, or to enable a candidate to sit for university examinations, etc.

The registration of births is still extremely incomplete in India, even in municipal areas.

In January 1939, Colonel R. S. Townsend, gave us a copy of a paper written by himself and Rai Bahadur Dr. Raghunandan Lall, M.B., B.S., on age determination of Indian girls in the United Provinces by x-ray demonstration of epiphysial union.

His paper was published in the *Indian Medical Gazette*, October 1939.

The present paper is a continuation of his work. He obtained from the Provincial Government a grant of Rs. 200 for this purpose and this investigation owes its inception to his enthusiasm and interest.

The requisite number of boys and girls could not have been obtained without the support of Miss Littlewood, inspectress of girls' schools, N. W. F. P., and the hearty co-operation of the head-mistresses and head-masters of schools in Peshawar, to all of whom we acknowledge our debt of gratitude.

The first problem was to collect an adequate number of scholars whose ages could be proved to be between 13 and 20.

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